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Proceedings
of the
Second National Research Conference
on
ANAPLASMOSIS IN CATTLE

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Oklahoma A. & M. College

Stillwater

February 18 and 19, 1953

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NOTE: The titles marked above with an asterisk (*) are reproduced from manuscripts submitted by the authors at the time of the conference. Others are from notes made during the conference by a representative of the Editorial Office of the Oklahoma Agricultural Experiment Station. These statements indicate the general trend of the discussions, but are not verbatim transcriptions.

AGENTS OF TRANSMISSION OF ANAPLASMOSIS AND THEIR CONTROL

(Summary)

D. E. Howell
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Stillwater, Oklahoma

The first proven vector of anaplasmosis was Boophilus decoloratus, in 1912. Then, in rapid succession, 15 species of ticks in 6 genera were incriminated, involving transmission from stage to stage, interrupted feeding of the same stage by males and females, and trans-ovarian. Insect species definitely incriminated include 9 of Tabanus, Stomoxys calcitrans, and mosquitoes. Epidemiological evidence indicates that hornflies (Siphona irritans) may be a vector. There is a good chance that any insect which draws blood and quickly goes to another animal to continue feeding may at times spread anaplasmosis. Such insects would include black flies, chrysops, biting gnats, and possibly house flies feeding on open wounds.

On one Oklahoma ranch having 7,000 animals, hornflies appeared to be the only insect involved. There was no laboratory evidence, but incidence of the disease dropped when vigorous hornfly control measures were taken. Mosquitoes and stable flies are important vectors only where present in large swarms.

Almost complete control of ticks has been obtained by spraying every three weeks with toxaphene 0.5% and BHC 0.015%. Only the hydrocarbons which give rapid kill are effective; those which do not kill immediately give the tick time to bite and transmit the disease before being killed.

Use of a treadle sprayer on the route taken by animals to water has been helpful in control of ticks and horseflies. This device is most effective against insects which feed on the backs of animals, and is not effective against those which feed on the belly, legs and udder.

For hornflies a routine spray every three weeks is effective; and in ranges where there are no trees rubbing posts give equally effective protection.

Against horseflies, pyrethrum and a synergist (piperonyls or sulfoxones) is effective, but the materials are expensive and not feasible for use on range cattle.

(Discussion)

Porter: Most Alabama cases have been traced to animals shipped into the state. Carriers are found in Alabama herds, but the disease does not seem to spread to other animals.

Muth: We think D. andersoni must be the vector in Oregon. The disease follows the emergence of ticks in about three weeks.

Oglesby: Most of ours in Louisiana have been traced to horse flies.

Muth: We have heavy Tabanid infestations, but there appears to be no correlation with anaplasmosis. Is it possible that humidity may be involved?

Schmidt: We have lots of anaplasmosis along the Texas Gulf Coast. No ticks have been incriminated. We consider the horsefly the principal vector in that area--the horsefly and perhaps occasionally a Dermacentor, probably albipictus.

Howell: Under laboratory conditions we have been unable to obtain transmission by horseflies held more than seven minutes between bites. It has been difficult up to five minutes, but easy at two minutes. I wonder, too, why there is little transmission by horseflies on the West Coast, in Australia, or in South Africa.

Muth: They are a different species.

Howell: In our experience Tabanids transmit anaplasmosis in Oklahoma, but do not in California. It seems unlikely that the difference in species is the whole story.

CONTROLLING ANAPLASMOSIS BY USE OF BIOLOGICS*

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Many attempts have been made to immunize susceptible cattle against anaplasmosis. All successful attempts depended upon premunition with either Anaplasma marginale and A. centrale (Theiler^{1/}, Cordier and Meneger^{2/ 3/}, Omlin^{4/}, Sergent, Donatien, Parrot and Lestoquard^{5/ 6/} or with attenuated forms of A. marginale (Lignieres^{7/} and Sergent et al. ^{5/ 6/}). All methods of premunition

* The material presented here under this title is from a manuscript entitled "Studies on the Use of Biologics in the Control of Anaplasmosis," by C. C. Pearson, W. E. Brock, and I. O. Kliwer, Veterinary Medicine, Vol. XLVIII, No. 11, pp. 435-37, November, 1953. This manuscript was developed from Pearson's original paper and covers the same material.

which depend upon introducing the living organism into cattle to produce a nonfatal form of the disease have one serious fault: They produce the carrier state in the vaccinated cattle.

Noting that no natural immunity exists in cattle and that an active immunity or tolerance is developed in animals that recover from the disease, it should be possible to develop a similar condition in animals by vaccination without the undesirable carrier state always present in animals that recover from the disease.

The authors have unsuccessfully attempted to produce killed organism vaccines which would produce immunity without producing the carrier state in cattle.

During the past five years, or more specifically beginning early in 1947, vaccination studies have been carried on at this station. During this time the tests have involved the use of one hundred twenty-five susceptible cattle and some fourteen different vaccines.

While the results reported in this study are negative, it is felt by the authors that a report of such results is important to prevent expensive repetition in research.

EXPERIMENTAL PROCEDURE AND RESULTS

It should be noted that due to the age and good general condition of the animals no death loss occurred during this series of experiments. The animals were all good quality Hereford steers and heifers and were all ranch raised. They were given no special care nor were they housed except in very extreme weather--not more than a few days in any winter. Their winter ration consisted of native pasture and from one to two pounds per head daily of 40% cotton seed or soybean pellets. They were given hay when the grass was covered with snow.

At the conclusion of each portion of the experiment the group was checked for carrier infection and all animals proved to be carriers.

Since all of the control cattle used in these experiments were treated in the same manner, the results from the total of the 18 control animals have been averaged and included in one control group. The average incubation period and low red blood cell count in this combined control group was 22.0 days and 2,850,000 respectively.

Vaccines 1, 2, 3, and 4

These four vaccines were prepared from spleen, spleen and lymph gland, blood, and bone marrow, respectively, collected from animals in the acute state of the disease. The solid tissues were ground and weighed, and a buffer solution added. This buffer was made of sodium dihydrogen phosphate and potassium dihydrogenphosphate plus glycerin. The pH of the diluent was 7.4. The material

was then passed through the colloid mill and a preservative of 0.5% phenol added. The blood used for vaccine 3 was prepared in the same manner as the other tissues except that it was not ground or passed through the colloid mill.

Ten susceptible 2-year-old animals were used to check each of these vaccines. Eight were given 40 cc. each of the vaccine at the first inoculation, and this inoculation was repeated 50 days later. Two of the animals were held as controls. Thirty days following the second inoculation, each of the test animals was challenged with an inoculation of 2 cc. of blood from a known carrier animal. All of the animals proved susceptible to the disease.

Incubation periods as well as the low red blood cell counts varied with individuals and groups. The averages were:

Vaccine	Incubation Period	Low RBC Count
1	22.4 days	2,177,000
2	24.6 "	1,852,000
3	27.7 "	2,540,000
4	24.7 "	1,960,000

Vaccine 5

Vaccine 5, a crystal violet blood vaccine, was prepared by adding the aforementioned buffer solution and 0.5% of crystal violet to blood collected from an acute case of the disease.

Twenty 2-year-old animals were used to check this vaccine. 40 cc. doses repeated in 20 days were given 16 of the animals, and four were retained for controls. Thirty days after the second inoculation all 20 of the animals were challenged with a 2 cc. dose of blood from a known carrier animal. All of the animals were susceptible to the disease. The average incubation period of the treated animals was 20 days and the average low RBC count was 2,900,000.

Vaccines 6, 7, and 8

These three vaccines were prepared from the following tissues, respectively: thymus gland and heart muscle, kidney, and lung. The tissues were collected as for vaccines 1, 2, 3, and 4, and were prepared in a similar manner except that eucalytol was used as a preservative.

A group of ten yearling animals was used on the thymus-muscle vaccine, using two as controls, and groups of five each on the kidney and lung tissue vaccines, using one in each group as a control.

40 cc. of vaccine was given each animal except the controls, and the dose was repeated in 21 days. Thirty days after the second inoculation each animal was

challenged with 2 cc. of blood from a known carrier and all animals proved susceptible. Average incubation periods and red cell blood counts were:

Vaccine	Incubation Period	Low RBC Count
6	22.4 days	2,910,000
7	23.5 "	2,220,000
8	22. "	1,930,000

Vaccine 9

Vaccine 9 was prepared by lyophilizing blood collected from an acute case of anaplasmosis.

Twenty yearling animals were used to check this vaccine. Sixteen received 20 cc. of the vaccine after the diluent was added and the same dose was repeated in 30 days. Four remained as controls. Thirty days after the second inoculation the animals were all challenged with 2 cc. doses of known carrier blood and all proved susceptible to anaplasmosis. The average incubation period for the vaccinated animals was 20 days and the low RBC average was 3,100,000.

Vaccine 10

Vaccine 10 was prepared by adding 1.25 gm. aureomycin to 40 cc. of diluent, and then adding 30 cc. of this solution to 200 cc. of freshly drawn blood from a known carrier animal. Twenty-four hours later 4 yearling cattle were given 40 cc. each subcutaneously and one remained for a control. Sixty days later the animals were all challenged with 5 cc. of known carrier blood and all were susceptible to the disease. The average incubation period was 21 days, the average low RBC count was 2,120,000 for the treated animals.

Vaccine 11

Vaccine 11 was prepared by adding 20 cc. of diluent to 400,000 units of crystalline penicillin G (potassium), and then adding this to 200 cc. of blood from a known carrier. Twenty-four hours later each of four yearling animals received 40 cc. of the vaccine, and one remained for control. The four animals receiving the vaccine contracted the disease from the vaccine and the average incubation period was 29 days and the average low RBC count was 3,130,000. There was in this instance a noticeable effect on the incubation period.

Vaccine 12

Vaccine 12 was prepared by administering 3.75 gm. aureomycin in 200 cc. diluent to a known carrier and drawing 200 cc. of blood from the animal 15 minutes later. Twenty-four hours later four animals received 40 cc. of this blood and one

remained as a control. The four animals receiving the vaccine contracted the disease, with the average incubation period being 31 days and the average low RBC count 4, 100, 000. Again the incubation period seems long, and in addition the disease seemed less severe.

Vaccine 13

Vaccine 13 was prepared by using 200 cc. blood prepared as for vaccine 12, to which was added 400, 000 units of crystalline penicillin G (potassium) in 20 cc. diluent. Twenty-four hours later four animals were given 40 cc. each of the vaccine and one remained as control. The four vaccinated animals contracted the disease with an average incubation period of 31 days and an average low RBC count 3, 750, 000. These results are very similar to those obtained with vaccine 12.

Vaccine 14

To determine the effect of gamma globulin (bovine origin) on the resistance of an animal to anaplasmosis, two four-month-old animals were used.

No. 235 was given 15 gm. and No. 236 given 20 gm. gamma globulin. No. 235 was given 5 cc. of known carrier blood 18 days later. The animal contracted the disease after an incubation period of 13 days. Animal No. 236 was given 5 cc. of carrier blood seven days following the gamma globulin. The animal contracted the disease after an incubation period of 11 days. These two incubation periods average only slightly more than half of the average of 22 days in the former controls used with vaccines 1 to 13. However, the course of the disease was normal for this age animal.

CONCLUSION

While we were able in a few cases to vary the incubation period and possibly the course of the disease, making it slightly less severe, we were unable to produce an active immunity or tolerance without at the same time developing in the animal the carrier state of the disease. Therefore, it appears that none of the methods of preparation described in this study will produce a satisfactory vaccine against anaplasmosis.

SUMMARY

Eight tissue vaccines were prepared from spleen, lymph gland, blood, bone marrow, thymus gland, heart muscle, kidney, and lung. None produced immunity to anaplasmosis.

Lyophilized whole blood from a bovine infected with acute anaplasmosis did not produce immunity against anaplasmosis.

Aureomycin added in vitro to anaplasmosis carrier blood prevented infection when the treated carrier blood was injected into susceptible cattle, but did not produce immunity against anaplasmosis.

Penicillin added in vitro as above with aureomycin did not prevent anaplasmosis infection when injected into susceptible cattle.

Neither aureomycin nor penicillin added in vivo, with the blood drawn 15 minutes following the administration of the antibiotic, prevented anaplasmosis infection.

ACKNOWLEDGEMENTS

All of the vaccines used in this study were prepared by Dr. Herman Farley and Dr. Lon E. Foote, formerly of the Oklahoma Veterinary Research Institute staff at Stillwater, Oklahoma.

The Gamma globulin (bovine origin) used in these experiments was furnished by Dr. M. A. Schooley, Director, Veterinary Research Department, The Armour Laboratories.

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(Discussion)

Christensen: It all comes down to the question, "What is the anaplasma?" Can you produce immunity if it's not a living organism?

Schoening: We have had several animals lose the carrier state as indicated by inoculation with blood of the previous carrier. The blood of these recovered animals had no protective power. Nor was the previous carrier immune; it reacted after having lost the carrier state.

Brock: We have one animal which has gone 293 days without relapsing to the carrier state, after being treated with aureomycin. She gives a +4 reaction on the complement-fixation test.

Splitter: We have four carriers which have been freed of anaplasma bodies; they will be discussed in Dr. Miller's paper.

Pearson: No complement-fixation tests have been made on our vaccinated animals to date.

Christensen: Apparently we must have an organism present in order to produce active resistance. We have made little progress by passage through animals. Do we have Anaplasma centrale in the United States?

Schoening: Apparently A. centrale blood vaccines are used routinely in South Africa.

Oglesby: Does a strain of A. marginale increase in virulence? Does progression through several animals seem to decrease the virulence?

Pearson: It is more a question of the resistance of the host. There has been a fairly uniform pattern in our own animals, but there has been less uniformity among animals of varying ages and conditions. We feel there is less variation in the anaplasma than in the animals.

Unknown: There is laboratory evidence of variation.

Miller: A vaccine of short duration, three or four months, would be useful in many areas. Let's not discard the possible value of using a dead vaccine.

Howell: It would need to be effective for six months where ticks and horseflies were vectors.

Christensen: Maybe we should consider the possibility of some other approach.

Schmidt: We should keep the age of the animal in mind when comparing reactions to drugs or vaccines. The test animal should be at least three years old; a young animal can regenerate the blood faster.

Christensen: I'd suggest that vaccination by vectors occurs continuously in cattle native to an anaplasmosis area, so the native animals must be virtually all carriers. Calves could become inoculated without showing the clinical symptoms. Then there is a flare-up when cattle come in from the outside.

Schoening: Should attention be given to the possibility of making a vaccine from macerated ticks, as was done with Rocky Mountain fever?

Howell: Dr. Boynton did that several years ago; the results were not particularly promising. However, the organism can be seen in the red blood cells in ticks up to 24 hours.

Christensen: We need to study whether the organism can move to one cell from another.

Schmidt: That would involve a question of the pH of the blood, etc., as they affect the possibility of transfer. In such studies, we do not always duplicate the internal environment of the animal.

Muth: Could it be done in the living animal?

General: If we could cultivate the organism, we might have the answer to the possibility of attenuation. --We get transmission in the serum because it is difficult to remove the red blood cells entirely. --We have had no effect from serum where we were sure there were no red blood cells. --What is the possibility of large doses of serum? --Inoculation of young animals might result in an embargo on cattle. We could pre-immunize all breeding stock, but not if it were going to be shipped.

Christensen: Animals inoculated with centrale become carriers for centrale but are protected from marginale; it might be done in young animals.

Schoening: Does centrale exist in the United States? Blood brought from South Africa would also bring other diseases. Also, centrale blood may not be inoffensive under our conditions; there will be no room for isolation of it at Plum Island for a long time to come.

General: Biological control is one of the main problems to be solved.

SEROLOGICAL DIAGNOSIS OF CARRIER ANIMALS OF
ANAPLASMOSIS BY MEANS OF
COMPLEMENT-FIXATION

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For a number of years the Bureau of Animal Industry has been engaged in a study of the application of the complement-fixation test to the diagnosis of carriers of Anaplasmosis. Early work by Reese and Mohler in 1934 showed that it was possible to demonstrate specific complement-fixing antibodies in serums of carrier animals, using an antigen prepared from ticks which had engorged on infected animals (Ref. 1). However, the difficulty in preparing antigen from ticks precluded this method as one of practical significance.

In Veterinary Medicine of April, 1949 and July, 1949, work was reported on the complement-fixation test for serum diagnosis of anaplasmosis and the production of an antigen for anaplasmosis complement-fixation tests. These articles were by Mohler, Eichhorn, Rogers, Mott and Gates of the Pathological Division of the Bureau. In these two reports the second phase of our studies was begun and these were stimulated through work done in the Army Medical School in Washington, D. C., by Kent and Rein on the serum diagnosis of malaria in human patients by means of the complement-fixation test. The same problem was present in soldiers returning from abroad with regard to carriers of malaria, as is seen in the problem of anaplasmosis in carrier animals. The question of diagnosis was of prime importance.

Using technics developed by Kent and Rein with some modifications, in the preparations of antigen and the techniques of the complement-fixation test as developed by Kent and Rein, very encouraging results were obtained in the diagnosis of the carrier animal in anaplasmosis. In these two papers techniques are reported on the production of antigen and the technique of the test itself. In the meeting held in Washington in February 1948 on the subject of anaplasmosis, these details as were then available were reported.

It is the purpose here to briefly review the progress of the work since the meeting in Washington in 1948. Reference is first made to the two papers which appeared in 1949 and using the modification of a method described by Heidelberger and Mayer, quite satisfactory antigens were prepared. Quite a large amount of this material was prepared for future use. Its stability was to be studied and this proved to be quite good. It was felt that the problem of antigen production as to preparation and production, was on a rather sound basis. However, as the original supply of antigen became reduced, production of antigen was again resumed in 1950. Difficulties were encountered in regularly preparing a suitable antigen and in one series of tests only about 20% of the antigens were satisfactory. Later

production increased the number of suitable antigens but it was apparent that information needed to be developed which would lead to methods whereby satisfactory antigens could be produced regularly.

Since 1950 we have been largely concerned with studying the various factors involved in the preparation of antigen with the hope that information would be developed which would lead to the production of a regularly satisfactory antigen. Progress has been made in this field but we are not yet in a position to say that we have arrived at the desired goal. Further research on this problem must be continued, which we hope to do.

I would like to refer very briefly to the efficacy of the complement-fixation test. We have had at Beltsville an experimental herd of cattle infected with anaplasmosis in which the status of each animal is definitely known, and in addition, of course, we have a herd of negative animals. Much serological work has been done on this anaplasmosis herd. Bleedings have been taken at regular intervals and have been tested, and through this means it has been possible to estimate the reliability of the test in this herd. With various antigens used, the efficacy of the test has been over 90%. It has been possible also in our antigen production studies to follow the serology of the animals before infection and after. Specific complement-fixing antibodies are demonstrated usually 10 days to 2 weeks after the animals have been exposed.

In our anaplasmosis herd two animals over the years lost their carrier state. Both of these animals were in the herd for some years and had given consistently positive reactions to our complement-fixation test. Sera from both animals, however, eventually became negative to our complement-fixation test and following this samples of blood from these two animals were inoculated into two normal, splenectomized calves. Both of these inoculated animals remained normal. This indicated that some animals may lose their carrier state and such animals may give a negative reaction to our complement-fixation test. This also furnishes further information on the specificity of the test.

Cooperative work has also been carried out with the Livestock Sanitary Service of the State of Maryland on the efficacy of the complement-fixation test for anaplasmosis. The results of this work were reported at the 53rd Annual Meeting of the U. S. Livestock Sanitary Association by Gates and Mohler of the Pathological Division of the Bureau, and Poelma and Hastings of the Maryland Livestock Sanitary Service. In this study, a total of 557 herds of cattle were tested by Complement-fixation in 22 counties in the State. Ten of these herds located in the Eastern shore were selected as particularly suitable for use in determining the efficacy of the complement-fixation test for the detection of carriers. Five of these herds had a history of infection with anaplasmosis. The ten herds consisting of 196 cattle were tested by complement-fixation: 57 were positive, 20 suspicious, and 119 negative. Two of these herds gave negative reactions to the tests and the remaining 8 herds contained animals that gave positive, suspicious, and negative reactions.

In order to test the efficacy of the complement-fixation test, the following procedure was adopted: An animal giving a positive reaction in a herd was selected at random. Blood from this animal then was inoculated into a splenectomized calf. A number of negative animals in the same herd were bled and the blood pooled and inoculated again into another splenectomized calf. In several instances, an animal giving suspicious reaction to the complement-fixation test was bled and this blood injected into a splenectomized calf. The negative animals in this herd were then bled, the blood pooled and injected into a splenectomized calf. The results of this investigation showed that of the nine splenectomized calves that were inoculated with the blood from random-selected animals reacting positively to the complement-fixation test, seven developed acute cases of anaplasmosis. Of two calves inoculated with the blood from animals giving suspicious reactions, one developed an acute case of anaplasmosis. The other calf which was inoculated with pooled blood from three suspicious reactors did not develop the disease. All ten calves that were inoculated with the pooled blood from the negative reactors to the complement-fixation test (76 animals) also failed to develop anaplasmosis.

In the meeting in Washington in 1948, in reporting the preliminary observations on the complement-fixation tests, it was urged that other States interested should take up the matter of complement-fixation and continue the development of improved techniques. Dr. Boynton, of California, who attended the Washington meeting, became quite interested in the serological diagnosis and arranged to do some testing in California with antigen prepared by the Bureau. A considerable number of samples were tested in California by Dr. Boynton and his group and these samples, in turn, were also tested by the Bureau in Washington. Likewise, Bureau samples were forwarded to Dr. Boynton for his testing and the agreement between the two laboratories was very close. Dr. Brueckner and his group in Maryland have also undertaken research work in the serological field which he will no doubt report on at this meeting.

It is felt that this whole matter should receive the attention of those engaged in research on anaplasmosis, since the problem is quite a complex one and the more people that can work on the techniques of a test, particularly antigen production, the sooner will be the development of needed information.

Certainly there are other methods of preparing and improving the antigen and this can only be brought about through research on quite a wide scale. We also believe that additional work along the lines reported by the Bureau and the Maryland workers on the efficacy of the test should be continued. In other words, it is felt that there are two problems that still must receive attention:

- 1) Study on methods of the production of antigen, and
- 2) Further tests on the efficacy of the complement-fixation test.

When these two points have been firmly established, then we will be in a position to come before the industry and say that we do have a method of diagnosing the carrier state of anaplasmosis by serological means that can be applied on a field basis.

February 9, 1953

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February 9, 1953.

ANTIGEN PRODUCTION

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Anaplasmosis antigens are made from blood of animals in the acute stage of the disease. Carrier blood is usually employed as source material and a number of serial passages made in splenectomized calves. Numerous modifications of plan may be used by pooling carrier blood and using more than one splenectomized calf for each passage. Mature cattle with or without spleens may occasionally be used as passage animals.

Most good antigens originate from splenectomized calves having a relatively high parasite count. This usually requires two passages with large infective doses. In the beginning many passage animals died soon after inoculation as a result of large doses of acute blood. This may be avoided by using washed packed cells for the inoculum. In this way most of the toxic material is removed by the washing process. When the parasite count reaches its maximum all the blood from each passage animal is collected in sodium citrate and processed immediately. The serum is removed, the cells washed in cold saline and added to a saturated solution of carbon dioxide. The resulting precipitate is washed in cold distilled water, the volume adjusted and the pH brought up to 6.5. The resulting product is then lyophilized in ampules containing one cc. amounts and stored at -70°C . for future use.

Most of the early antigens demonstrated a high degree of antigenic value and therefore it was thought that good antigens could be produced at will. Several years later when antigen production was again resumed, it was found that this conclusion was false.

Series G antigens were produced in 1949 - 1950 by making 12 serial passages in splenectomized calves. In this series blood from one Florida carrier was used as source material and the infective dose was 1 cc. of packed cells or its equivalent in whole blood, per lb. body weight. One splenectomized calf was used for each passage. The CO_2 method of lysing the red cells was employed. Only 20% of these antigens demonstrated any antigenic value.

Series H antigens were produced during 1950 and 1951 by making 19 passages in splenectomized calves. In this case blood from a Louisiana carrier was used as source material. One and in some cases two splenectomized calves were used for each passage. The infective dose and the method of lysing cells were the same as that employed for the previous series. Seventy per cent of these antigens gave a satisfactory titer.

Series I antigen was produced 1950. Blood from one Louisiana carrier was used for source material. One passage in a splenectomized calf was made, using the same infective dose and method of lysing the red cells. This resulted in an antigen which was insoluble following lyophilization. This served to further complicate the antigen production problem. However, it was soon discovered that the reason for the insolubility of the antigens was faulty lyophilizing technique and therefore could be easily corrected.

Series J antigens were produced during 1950. Blood from a Louisiana carrier was used as source material. Five passages were made in splenectomized calves, using from one to three animals for each passage. Small lots of antigen were made every two days from each animal, beginning before inoculation and continuing until death or recovery. One hundred and ten antigens were produced in this series. Only a few of the antigens had any antigenic value. All the good antigens were produced at or about the time the parasitized cells reached their maximum.

Series K antigens were produced in 1950 by making 3 serial passages in splenectomized calves and whole cows, using pooled packed cells from 10 carrier animals as source material. Two splenectomized calves were used on the first passage. Five splenectomized and one whole cow were used for the second passage and a like number for the third passage. This method resulted in reducing the incubation period for carrier blood from approximately 15 days to 8 days. It required only 6 days for the bodies to reach their maximum as compared with 7 to 9 days in the usual case. Ten antigens were produced in this series, nine of which gave a satisfactory titer.

Series L antigens were produced during the latter part of 1952. Blood from a Florida carrier constituted the source material. Ten passages were made in splenectomized calves. One-fourth cc. of packed cells per lb. body weight was used throughout this series for inoculation. This quantity of acute blood will produce acute anaplasmosis in 24 hours and at the same time result in a great saving of cells which can be utilized for antigen.

During 1950 careful studies and titrations were made on 140 different antigens. It was found that most of the good antigens resulted from splenectomized calves, weighing 500 or more lbs., free of Bartonella infection, having a one day incubation period and 50 to 80% of the red cells parasitized. It was also found that lyophilization and storage at -70°C . was the best method of preservation.

Some recent work was done in which numerous modifications were made in the method of processing the blood. The results indicate that 4 cell washes are superior to 6 washes. In washing the precipitate, it was found that a centrifugal force equivalent to 20,000 times gravity (13,000 R.P.M. on a SS-1 model angle Servall Centrifuge) is necessary in order to throw down all the antigen and make it possible to remove the supernatant without losing any antigenic material. It was also found that in some cases a centrifugal force equivalent to 225 times gravity

(1000 R. P. M. on a No. 2 International Centrifuge) will remove most of the coarse sediment and result in the elimination of the anticomplementary factor. It is planned to continue research on antigen production and it is hoped that in the near future satisfactory antigens will be produced consistently.

(Discussion)

Groth: The problem lies in the unknown and unsuspected carriers. We must be able to identify them if we are to eradicate anaplasmosis.

Price: Maryland has four antigens, one of which is also being used at Pawhuska. Nine satisfactory antigens have been prepared at various laboratories, using the Maryland method. In tests run at Pawhuska, using one of the Maryland antigens, the Pawhuska staff got a 69% accuracy.

Miller: Forty-five of 46 positives were correct at Louisiana.

Schmidt: Australia prescribed a method of testing King Ranch cattle for carriers. It was not feasible for 250 cattle because it used splenectomized calves.

Davis: Meat inspection can spot carriers from areas not previously known to have anaplasmosis. There is some question about diagnoses made without using blood smears.

Mohler: Animals known to be affected with anaplasmosis do sometimes lose their carrier status, and are not immune to reinfection. (NOTE: A manuscript "The Use of the Complement-Fixation Test for the Titration and Test of Serum for Anaplasmosis" has been prepared by Dr. Mohler.)

THE USE OF ANTIBIOTICS IN THE CONTROL OF ANAPLASMOSIS

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Splenectomized calves are used as the basis of much of our experimental work. We believe that the almost constant increase in the percentage of infected R.B.C. is the test area of choice. Drugs or antibiotics are evaluated for their anti-anaplasma properties by their ability to modify or alter the rate of red cell infestation. Dosages are increased to nearly lethal levels where necessary.

At L. S. U. we embarked on the testing of antibiotics after unsuccessfully working on anti-malarials. For our first tests we selected two wide spectra antibiotics: aureomycin and terramycin. Bearing in mind that the classification

of the anaplasma is by no means certain, it was felt that an agent with a wide range of activity would be more likely to be effective than an agent which is almost pinpoint in selectivity.

Aureomycin was administered at the rate of 5 mg/lb. IV, and terramycin at the rate of 2 mg/lb. IV to 32 calves. All the treated and control calves were inoculated from common carrier source which has the most virulent strain of anaplasma that we have yet encountered.

The increase of anaplasma infestation was immediately halted and declined after a single treatment with aureomycin and regardless of the point of treatment --5 to 60 per cent of infected cells. This was only true in 25 per cent of the cases treated with terramycin. In the remaining 75 per cent the increase in cell infestation was halted, but remained almost constant for 2 to 3 days before declining. One hundred per cent recoveries were obtained with the 32 calves treated with these antibiotics, compared to 60 per cent fatalities in 26 untreated control calves.

- 3 Slides -

Chloromycetin was also screened at 50 mg/lb. divided dosage given orally, with no results. However, when administered at the rate of 100 mg/lb. IV in single or divided doses, a temporary inhibition was obtained followed by an increase in the rate of infestation, which often reached extraordinary levels.

Lower dosage levels of aureomycin and terramycin were investigated. A 50 per cent reduction of the original dosage level gave the same results. This is important in view of the current discussion on the reduction of antibiotic dosage levels. Further reduction to 10 per cent levels also gave an inhibition but 5% also gave temporary inhibition such as obtained with chloromycetin. We therefore have 3 antibiotics with definite activity against the anaplasma.

- Slides -

We attempted to eradicate the anaplasma from adult carriers of this disease. Aureomycin was used at the rate of 2 1/2 grams IV daily for 20 days and terramycin at the rate of 1 gram daily for 16 days. The carriers were still infective after 10 days of treatment, but not at the end of treatment. After 60 days, the bloods of these animals were again infective. It is apparent that there is a focus of anaplasma infection that is outside of the blood stream and was not reached by the treatment.

Subsequent work by Splitter at Kansas using increased dosage of terramycin have kept a carrier free of infection for 200 days to date, after treatment. Similar work at Louisiana, using Splitter's treatment schedule with aureomycin, has kept carriers free of infection for 60 days after the end of treatment. It would appear that there is a real chance that we may be able to eradicate the infection in carriers.

With this background of experimental data, the question arises as to the value of terramycin and aureomycin in the treatment of field cases. We were unable to conduct an evaluation under close observation, but we did treat 132 clinical cases. Two and a half grams of aureomycin or 1 gram of terramycin were used IV--this dose being arbitrarily doubled for animals over 1000 lbs. in weight.

We found that 72 per cent of all cases treated were presented for treatment when they had less than 30 per cent of their hemoglobin remaining (4. grams per 100 cc blood). Many of these animals had been visibly ill for 4 to 5 days, were emaciated, jaundiced, weak, and frequently unable to rise. These animals had no fever and often only a low percentage of infected R.B.C. We do not feel that specific antibiotic therapy was indicated in these cases. The antibiotic will inhibit the growth of the anaplasma and we hope ameliorate the symptoms. In these cases, the active growth of the anaplasma has already ceased and the symptoms we hope to ameliorate are present in their full force.

By contrast, in those cases that were treated above 4.0 grams of hemoglobin per 100 cc. of blood, 100 per cent recoveries were obtained in the aureomycin treated cases and 85 per cent of the terramycin cases. Probably because of the sporadic nature of the disease in Louisiana, the owner is dealing with one or two cases, and hence diagnosis is delayed. However, these antibiotics may be of real value where there are herd out-breaks and vigilant and intelligent observation by the owner may enable the cases to be treated early.

The effective use of these antibiotics in the treatment of field cases is dependent on their administration in the early clinical or patent phase of the disease. Work at Louisiana has shown that treatment early in the patent phase results in immunity. However, Foote has also shown that treatment in the incubation or prepatent phase only results in prolongation of the incubation period. Therefore, there is a very definite limitation on the timing of the treatment and it rules out indiscriminate prophylactic treatment of herds threatened with an anaplasmosis outbreak.

Miller discussion

(Discussion)

Miller: Our recovery rate with aureomycin treatment has been 80 per cent where animals were above 4 grams hemoglobin when treatment was started. Recovery with only supportive treatment has been 50 per cent.

Splitter: Work at Kansas corroborates Miller's findings at Louisiana.

Brock: Foote's animals after 60 days showed a negative phase lasting from 19 to 60 days.

Miller: The antibiotics inhibit the infestation, but the animals develop the anemia; hence there is need for supportive treatment.

Gates: We have treated three cattle with 4 mg. of aureomycin hydrochloride per pound of body weight given every six hours for 12 doses. One animal received 51.6 gms., another 32.8 gms. and the third 15.8 gms. All three of the aureomycin treated cattle transmitted anaplasmosis to splenectomized calves when subinoculations of 140-146 cc. of packed red blood cells were made ten days following the treatment. (NOTE: A manuscript "The Effect of Aureomycin on Anaplasmosis Infection" has been prepared by Dr. Gates and Dr. Mohler.)

DISCUSSION OF CHEMOTHERAPEUTICS OTHER THAN ANTIBIOTICS*

Oglesby (discussion leader)**--

1. In the mid 30's there were reports on various arsenicals--mercurochrome, aricyl, quinine and many trade name hematinics.

2. At the 1940 national meeting on anaplasmosis, most of the talk on treatment revolved around Dr. Boynton's recent report of almost miraculous success with sodium cacodylate.

3. At the 1948 meeting antimalarials, particularly atabrine, received quite a little comment.

4. More recently aralen dihydrochloride and chemozine came in for their share of use and considerable work was done with them.

According to the literature, all of these past studies have been made using mortality as a criteria. We now know that mortality alone is far from a satisfactory way of measuring the effectiveness of any drug against anaplasmosis. In many cases we have field reports showing almost miraculous results when the accurate differential diagnosis had never been made. (One glaring example was two cases treated in a town in Southern Oregon and the report went on to say that the animals had made complete recovery six months later. There was nothing about the clinical picture that fitted classical anaplasmosis and the report indicated the diagnosis was not confirmed by blood examination.)

* At this point, by prior agreement, the discussion following Miller's paper was turned to the subject of chemotherapeutics other than antibiotics. This discussion replaced a paper on that topic.

** From notes made by Dr. Oglesby the evening following the discussion.

5. During the past couple of years, most work has been done on antibiotics, as just discussed.

6. We studied at our Station, aralen dihydrochloride and chemozine to see if they had any effect upon the growth of the anaplasma body. In our experience, they were absolutely worthless. The infection continued to progress and the pattern paralleled exactly that seen in the untreated animals.

7. We must keep in mind that the clinical picture does not parallel the changes which take place in the blood picture. Animals studied closely will show that some times the blood picture follows quite a definite sequence from the standpoint of decrease in red blood cell count, decrease in hemoglobin, the decrease in R.B.C.'s infected without the animal breaking with visible clinical symptoms. In other instances this sequence takes place and the clinical syndrome comes on after the blood picture is beginning to improve. Nevertheless, many of these animals eventually succumb and some with what we commonly call acute anaplasmosis.

Study of the blood picture shows essentially no immature erythrocytes.

8. It would appear that there are at least two or three fundamentals which must be kept in mind, and probably others which have not come to light, when studying effectiveness of drugs used against this disease. Here are two or three:

a. The causative agent, whatever it is, is associated with the erythrocytes.

b. A little over 24 hours after a tick has engorged on infected blood, the anaplasma body as such has been destroyed and can be no longer demonstrated. However, some of these ticks remain carriers for many months. Further, in the recovered animal, it is difficult to demonstrate marginal bodies with regularity. Still, extremely small amounts of blood from this animal carries the infection. Do these two observations suggest an ultramicroscopic form or phase of the causative agent?

c. How or is the response of the anaplasma tied to the chemical formula of the material used? (Some very sketchy observations indicate that perhaps there is an affinity of certain chemical formulae for the causative agent of anaplasmosis.) Certainly, the bacteriologist can give us some excellent explanations as to why certain types of organisms, for example, grand positive as compared to grand negatives, are sensitive to certain antibiotics and sulfa compounds, etc. Is it not possible then for this to be a definite consideration in the study of anaplasmosis?

In the discussion that followed, there was no particular recommendation for any type of therapy beyond the use of blood. All suggested that blood was an extremely useful tool and that it should be administered in large quantities, not less than a gallon and preferably in quantities of two gallons to mature animals. Dr. Schoening suggested that those working with chemicals, consider working with the chemical Biological Coordination Center, Dr. Kirner, Director.

(Discussion)

Miller: We are now using two new chemicals with new solvents; they are closely related, but different in their activity.

Christensen: Don't sell transfusion short. Blood from slaughterhouses could be used as a bank, using one to two gallons for a transfusion. We have gotten away from chemotherapeutics.

Oglesby: There is a possibility of using periodical blood smears to detect early infection.

Brock: We need to study the life cycle as related to temperature suppression after the use of antibiotics.

Pearson: We do need more basic knowledge of the organism. I'd like to offer the Pawhuska facilities for testing vaccines.

Splitter: We have had no success with antimalarials.

Hoyt: Antihistaminics might be added to blood for transfusions, to eliminate side reactions.

Schmidt: Where does the organism go when it disappears from the blood of a cow?

SUMMARY OF CONFERENCE

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The conference has brought out, not so much what we know, but what we don't know. There seems to be more positive information on vectors than on any other subject, yet Dr. Howell said they are not fully understood.

We ought not learn to live with a disease, but rather learn to eradicate it--that's the philosophy we've developed in the United States.

I am much interested in the work at Pawhuska. The results of their research to date has been discouraging, but I trust they will continue to work and will come up with ideas for elimination of anaplasmosis. If any of you have ideas that might be helpful, I suggest that you give it to the gentlemen at Pawhuska.

Dr. Christensen touched on a fundamental matter when he remarked that we need more basic information about the organism; that probably is the key to the whole situation. In the past, agricultural research usually has started with the obvious and work back to the nature of the material involved, before coming up with an answer.

Dr. Schwartz has remarked that he could distinguish a central as well as a marginal object. If so, we may have centrale for use in immunization. However, we must have basic knowledge in order to grow the organism outside the animal body.

As far as possible, we need to help the general practitioner by putting in his hands the results of tested treatments.

Finally, I suggest that this group get together more often to exchange ideas and thereby stimulate the program to go faster.

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